Proceedings of the Royal Society B: Biological Sciences

Electronic supplementary material to
DOI: 10.1098/rspb.2017.0369

Consistent cooperation in a cichlid fish is caused by maternal and developmental effects rather than heritable genetic variation

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Origin and husbandry of experimental animals

Experimental *Neolamprologus pulcher* subjects in this study came from two sources: The test fish were F1 offspring of wild fish (F_0) captured in December 2012 in six of the eight distinct populations in Lake Tanganyika described in [1]. Upon arrival in our facility, the wild-caught fish were kept in single-population 400L-tanks until used for the breeding experiment. All other fish, dominant territory owner in the experiment and pairs producing the experimental clutches, stem from lab-stock populations of our animal keeping facility which were all descendants from fish caught in 1996, 2006, and 2009 near Kasalakawe, Zambia. Clutches for the helping test were obtained from pairs unrelated to the test fish. *Telmatochromis vittatus*, that were used as egg predator stimulus fish were also bred in the lab from wild parents and we housed them in two groups in 200L tank separated into two compartments. For the egg-predator presentations during early-life treatments a separate set of individuals (N=11, mean standard length (SL)=48.3±6.5 mm, range 39-63 mm) was used than for the helping tests (N=14, mean SL=44.9±10.3 mm, range 36-67 mm). All fish were kept in tanks within acclimatized rooms (24– 29°C), with artificial light from 08:00 to 21:00, and fed with Tetramin flake food on five days a week and once a week with thawed frozen food, containing Cyclops spp., shrimp, Artemia spp., and mosquito larvae). Test fish were fed with Tetramin 'Baby' food until they were tested at the age of approximately three months.

Experimental families and early-life egg predator treatments

Before being paired up, all wild-caught fish were weighed and measured. These were subsequently transferred to 60L breeding tanks equipped with a gravel layer, two clay flowerpot halves serving as breeding shelters and a filter. In total we set up 41 males with 79 females, but we excluded clutches of insufficient size and hatching rate (below 5 surviving juveniles to day 0). Whenever possible, males were paired up again with new females, but we had to exclude 2 males and 2 females from the experiment because they never spawned a clutch of sufficient size. Consequently, 3,175 individuals from 77 experimental families (39 males and 77 females) entered the experiment. One male died before he could spawn a second clutch, thus no half-sibling group could be produced. As soon as the fry became independent of brood care (day 0), which happened on average eight to ten days after spawning, we removed both parents, returned the female to the population tank and paired the male with another female in a different breeding tank. After two more days we caught and counted all the fry of a family and randomly assigned half of them to either of two early environmental rearing treatments following the procedure described in [2]. The fry were kept in their original tank, but we divided the tank with an opaque plastic sheet to make one compartment for each treatment group. Egg predator treatments were carried out as follows: First, a plastic container holding an individual *T. vittatus* was gently inserted and left in the tank for ten minutes. The control group received a sham treatment during which the container was lifted from the tank and reinserted, and we repeated this procedure after ten minutes to provide the same amount and schedule of handling to both treatment groups. Predator and sham treatments were performed on two randomly chosen days of a week. On three other days of the week both groups received the sham treatment so that in total each group of offspring was manipulated five times a week. After the last treatment on day 63 the early experience phase ended, and the fish were kept in the breeding tanks until day 85 (neutral phase).

Hierarchy phase, size measurements and selection of test subjects

The hierarchy phase started on day 85 and lasted for two weeks. On day 85 we measured the standard lengths (SL) of 1,331 fish in the egg predator treatment groups and 1,319 in the control treatment groups of the 77 experimental families (average SL of 16.3±2.7; mean±sd). Importantly, individuals raised with egg predator treatments did not differ in size at day 85 from the control group (ANOVA: $F_{1,2648}$=0.33, p=0.57), but offspring of the different populations did (ANOVA: $F_{5,2644}$=6.84, p=<0.001). However, the difference of the means of the largest and the smallest population of 0.9 mm is unlikely to have an important impact on the performance in the helping test. *N. pulcher* start to perform direct brood care in form of egg cleaning after reaching sizes of 10-15 mm [3]. Therefore we only used individuals larger than 15 mm for the tests. We also excluded the largest fish of each treatment group to avoid testing fish with dominance experience except in cases where there would have been less than six potential test fish left per treatment group. From all remaining individuals of a treatment group we randomly chose six individuals (i.e. twelve individuals per experimental family) as potential test fish and housed them individually in 20L test tanks (N=980, Fig. 1c in the main text). We assigned each potential test fish a 4-digit alphanumeric random code in order to blind the observer (CK) with respect to the treatment group and the half-sibling structure and egg predator treatment. *N. pulcher* only display alloparental brood care when they are subordinate [4]. Therefore we housed each test fish with an individually marked lab-stock fish that was approximately 1/3 larger for 14 days in order to let them establish a hierarchy. In this species, hierarchies are linear and size-based [5] and therefore the test fish was generally the subordinate except in cases in which they did not establish a hierarchy or the dominant evicted the test fish. We categorized the test fish's acceptance status by the dominant into five different classes (according to a previously established protocol by [6], Fig. S1) This was done by qualitatively evaluating the location of the test fish in relation to the dominant and the shelter, the test fish's use of the available space, and if it showed submissive behaviour towards the dominant during two minutes of observation. We preferentially chose fish that were classified as 'fully accepted' (N=28), 'accepted' (N=211), 'fully tolerated' (N=119) or 'tolerated' (N=22) before the first test (Table S1). However, if not enough fish were available, we also tested fish for which determination of the acceptance status was not possible ('not determined', N=10) or that were 'evicted' (N=9). For the second test we were not able to assess the acceptance status for 35 of the 86 already tested fish for logistic reasons, which we classified as 'not determined'. However, we included those individuals in our analysis to attain a sufficient sample size for the repeatability analysis.

Table S1: Proportions of individuals in the acceptance categories in the first and second test. Chi-Square tests provide information about whether fish with different acceptance status differed in the probability to perform helping behaviours. Note that no degrees of freedom are provided by the Chi-Square test as p-values were computed by Monte Carlo simulation.

First test		**egg cleaning**		**digging**		**defence**		**total**
		NO	**YES**	**NO**	**YES**	**NO**	**YES**	
fully accepted		17 (61%)	11	13 (46%)	15	0 (0%)	28	28
accepted		123 (58%)	88	83 (39%)	128	21 (10%)	190	211
fully tolerated		65 (55%)	54	60 (50%)	59	9 (8%)	110	119
tolerated		12 (55%)	10	15(68%)	7	3 (14%)	19	22
evicted		6 (67%)	3	5 (56%)	4	0 (0%)	9	9
not determined		8 (80%)	2	6 (60%)	4	2 (20%)	8	10
		231	168	182	217	35	364	**399**
Chi-squared test	**X^2**	**3.0**		**10.2**		**6.4**		
	sim. p	**0.696**		**0.066**		**0.251**		
Second test		**egg cleaning**		**digging**		**defence**		**total**
		NO	**YES**	**NO**	**YES**	**NO**	**YES**	
fully accepted		1 (33%)	2	0 (0%)	3	0 (0%)	3	3
accepted		4 (24%)	13	1 (6%)	16	2 (12%)	15	17
fully tolerated		4 (50%)	4	4 (50%)	4	0 (0%)	8	8
tolerated		2 (29%)	5	4 (57%)	3	1 (14%)	6	7
evicted		2 (40%)	3	2 (40%)	3	1 (20%)	4	5
not determined		25 (60%)	17	27 (64%)	15	7 (17%)	35	42
		38	44	38	44	11	71	**82**
Chi-squared test	**X^2**	**7.7**		**19.7**		**2.3**		
	sim. p	**0.160**		**0.0005**		**0.842**		

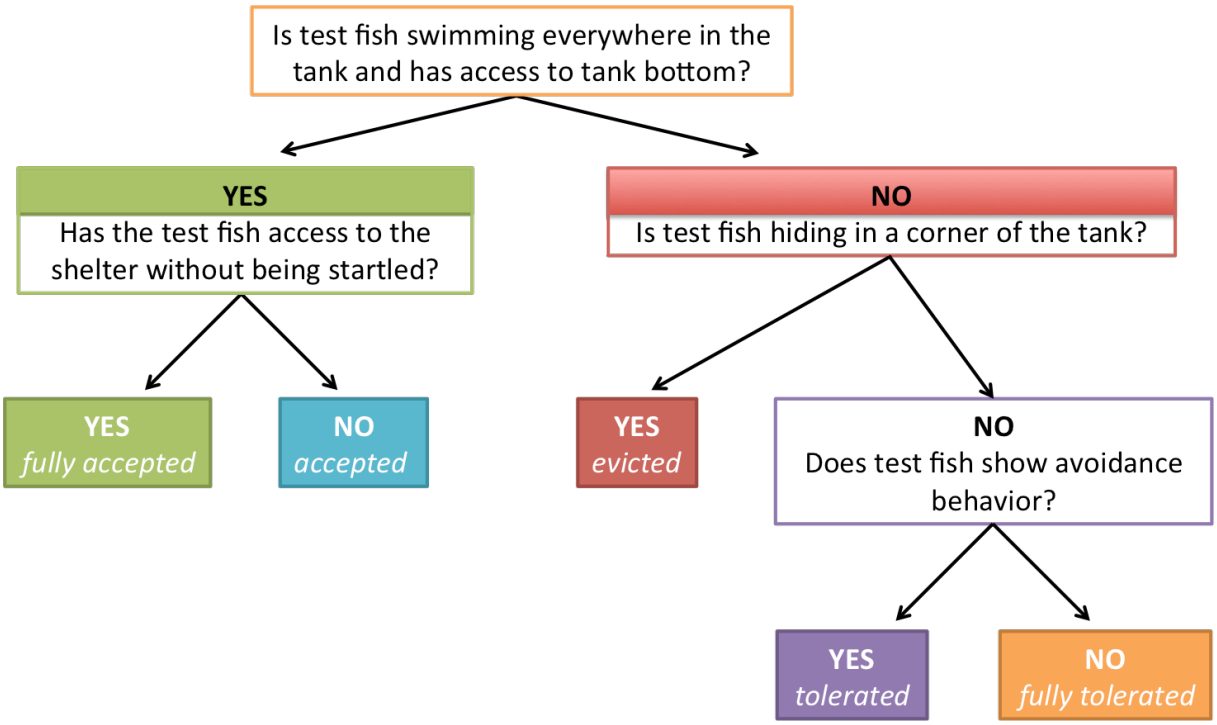

Fig. S1: Decision tree used for determining the acceptance status of the test fish during the hierarchy phase. Fish in all categories except 'evicted' had access to the shelter when sudden movements or noise startled them.

Behavioural tests

Before testing, the dominant was locked it into a vertical transparent tube of 13 cm diameter with a mesh lid, which prevented it from interfering with the helping task but, at the same time, ensured that social interactions between the two fish remained possible and the subordinate status of the test fish was maintained throughout the test. The box containing the dominant was placed in the back of the

tank (Fig. 1d in the main text), allowing social interactions between the test fish and the dominant, albeit of a somewhat limited repertoire. The test fish could join the dominant and show submissive behaviour (tail quiver, see ethogram in Table S2) towards it, whereas the dominant could approach aggressively, display threats and even direct attacks against the test fish through the transparent wall of the container. In most cases the test fish occupied the shelter after the removal of the dominant even if it had been usually chased out of it during the preceding hierarchy phase. On the test day a LED-light source was directed into the shelter to facilitate observations in the dark shelter. A 20-min period followed, in which the test fish could habituate to the light. Observations started either as soon as the test fish had stayed for 5 sec continuously in the shelter (in a pilot experiment this time span had been sufficient to enable the test fish to detect the clutch) or 10 min after the insertion of the clutch, whichever happened first. Using the event-logging software Observer (Noldus Information Technology) we directly observed the test fish' and the dominant's behaviour according to the ethogram in Table S2.

Table S2: Behaviours recorded in the helping test. Behavioural terms as used in the paper are given in bold. Single behaviours that constitute defence are given in italics. 'Type of variable' refers to the way the variable was modelled.

behaviour		type of variable	description
digging		dichotomous	removes sand from breeding chamber; takes up sand with its mouth/digs into sand with whole body
egg cleaning		dichotomous	nibbles on eggs; short, distinctive movements, can be gentle or vigorous
defence	*fast approach*	count	fast movement towards another fish, move-stop, no physical contact
	attack	count	sudden movement towards other fish with body contact (including ramming and biting)
	head down	count	body posture with tail fin higher than head, generally smooth transition from fin spread, usually similar stiffness as in fin spread
	bow swim	count	similar to attack but aggressor swims away and returns to other fish, head first; fast movement
	opercula spread	count	opening of gills, lowering of branchiostegal membrane, quick and sudden movement (flashing) mostly shortly before approach/attack
		sum of counts	fast approach + attack + head down + bow swim + opercula spread
submission		count	'tail quiver' display: trembling movement with tail or whole body; usually tail fin is directed to other fish, dorsal fin flattened; shown by subordinate, can be reaction to dominant approaching/attacking but also shown without any (re)action of dominant.
egg predator activity (*T. vittatus*)			average swimming activity of the egg predator individual recorded every 30 sec during the test (1=up to 25%, 2=26-50%, 3=51-75%, 4=76-100% of time spent active). A weighted average was calculated as the number of records in each of the categories 1-4, multiplied by the respective weight (1-4), divided by the total number of records.

At the end of the trial the egg predator and the clutch were removed, and the test fish was uniquely marked with visible implant elastomere (VIE) tags (Northwest Marine Technology, Inc.) and transferred back into its original tank. In total, we tested 454 fish from 75 different families (75 different females mated with 39 males). After having spent one week in their original breeding tank with the siblings of their treatment group (neutral phase 2, Fig. 1d in the main text), test fish were again housed for one week separately in test tanks with a different dominant individual (hierarchy phase 2). A second helping test was conducted with each of these individuals following the protocol as outlined above, but using a different egg predator individual. This provided us with a second measurement of cleaning and digging propensities as well as defence effort for this subset of

individuals, allowing us to assess its repeatability. The behaviours that were recorded in the helping test are listed in Table S2. We recorded helping behaviours (digging, egg cleaning and defence) and the test fish's social behaviour towards the dominant individual, which exclusively consisted of submissive behaviour.

Treatment of data before analyses

Before the analyses we excluded two types of 'non-responders': (i) test fish that never entered the shelter in the first 10 minutes of the helping test and thus were not able to perceive the eggs (N=54) and (ii) test fish that never left the shelter in the last ten minutes of the helping test and could therefore not perceive the egg predator (N=5). The dataset used in the analyses therefore contained 399 individuals of which 82 were tested twice (N=481 observations). The histograms of cleaning and digging counts show high zero inflation (Fig. S2), thus we dichotomized those variables (see main text).

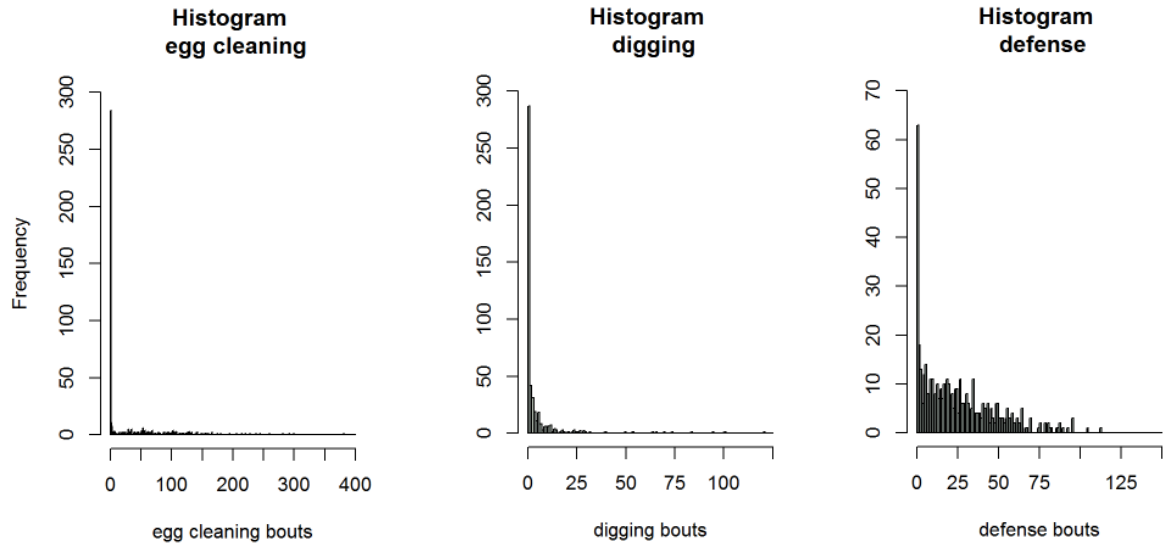

Fig. S2: Histograms of the counts of egg cleaning, digging (removal of sand), and defence against an egg predator individual during the helping tests.

Predictors of helping behaviours

Choice of predictors to be included as covariates

In the first step of the analysis, we selected a set of variables, which we expected to have a potential influence on helping behaviours (Table S3). We included (i) the early-life egg predator **treatment** as a covariate in the models because we hypothesized that test fish that were subjected to the treatment performed more helping behaviours than test fish in the control treatment. It is conceivable that the test fish's absolute size influence the motivation to help, even though the size range of all test fish was rather narrow (15 – 24.5 mm SL). However, the heritability of size at day 85 was very high (h2=0.97 (0.95, 0.98)) and we therefore decided to include (ii) a measure of **relative size** that also reflected

test fish's dominance experience in the natal group before the test. We assumed that the quality of the hierarchy between the test fish and the dominant could have an influence on the test fish's motivation to invest in helping, and thus we included (iii) the **acceptance status**. Furthermore, the (iv) **clutch size** that we presented in the helping test could be important for the test fish's motivation to clean or defend a clutch. We were unable to control this variable during the test because we had to use clutches that were spawned by the stock pairs on the same day or a day before the experiment. The available clutches were split in a way that each test fish received new, i.e. not already cleaned, eggs. We hypothesized that social interactions of the test fish with the dominant during the test could also influence the likelihood or the amount of help. Previous research suggested that *N. pulcher* subordinates that help less display more submission towards dominants as an alternative appeasement strategy that enables them to remain accepted in the territory [7]. Therefore we also included (v) the amount of **submission** displayed by the test fish towards the dominant during the helping test. Finally, we included (vi) **egg predator activity** in the defence model because we know from similar experiments, in which egg predators were presented to *N. pulcher* groups in the field, that more active stimulus fish are attacked and threated more often by the test fish (B. Taborsky unpubl. data).

Table S3: Covariates with potential influence on helping behaviours used in the models. The type of variable is printed in italics.

variable name	**description**
(i) treatment	part of experimental design with the aim to mimic environments differing in egg predation risk and hence 'demand' for help in a territory; *categorical variable* ('treatment' and 'control'); not used in digging model
(ii) relative size	proportion of smaller siblings the test fish had in its natal group; *proportion*
(iii) acceptance status	hierarchy between test fish and dominant as classified in section 'Hierarchy phase' above; *categorical variable* ('fully accepted', 'accepted', 'fully tolerated', 'tolerated', 'not determined', 'evicted')
(iv) clutch size	number of eggs contained in the clutch presented in the helping test; *count variable*
(v) submission	number of submissive behaviours test fish directed towards dominant (see ethogram Table S2); *count variable*
(vi) egg predator activity	activity of egg predator individual used in the second part of the helping test averaged over 10 min (see ethogram Table S2); *weighted average of categories* (1 to 4); only in defence model

Model selection

In the first step of the analysis we fitted GLMMs using the lme4 package V 1.1-12 in R [8] to test whether the potentially important predictors of Table S3 influenced helping behaviours. Since the data were hierarchically structured (treatment groups nested within dams, dams nested within sires, repeated measurements of some individuals, repeated use of dominant individuals, and, in the case of the defence models, repeated use of the same egg predator individuals) we included the identities of a test fish's father, mother, and treatment group, as well as those of their dominant partner. We aimed at using the same set of predictors for all helping-behaviour models, but for the defence model we added 'egg predator activity' as a fixed effect and the identity of the egg predator individual as a random effect. Due to convergence problems of the digging model we removed 'treatment' after an initial run that was done without 'acceptance status' since this variable did was not contained in the best model. After removal of 'treatment', the digging model selection including 'clutch size', 'relative size', 'submission' and 'acceptance status' could be performed without any convergence problems (Table S4).

Table S4: Selection of models containing predictors that are likely to influence **(a)** egg cleaning propensity, **(b)** digging propensity and **(c)** amount of defence. For cleaning and digging models, the identities of test fish, mother, father and dominant as well as the population of parental origin were included as random effects. 'Amount of defence' additionally contained the identity of the egg predator used in the test. Digging models only converged after removal of 'treatment', which we could exclude as an important predictor in a previous model. Only models of the confidence set (within a ΔAICc of 6, Richards et al., 2011) are shown, with estimates on the latent scale, except for 'acceptance status' (categorical variable with several levels). The sign of the estimates indicate an increase or decrease of the respective propensity or amount of helping behaviour relative to the intercept, i.e. each additional egg in the experimental clutch increases egg cleaning and digging propensity whereas each bout of submission decreases the propensities/amount. The selected models are printed in bold (see main text for model selection criteria).

	No	intercept	clutch size	test fish size	egg predator activity	treatment (control)	submission	acceptance status	df	AICc	delta	weight	mR2	cR2	nested
	18	**-0.33**	**0.34**	**NA**		***NA***	**-0.45**	***NA***	**8**	**647.360**	**0**	**0.43**	**0.066**	**0.238**	**---**
	20	-0.33	0.33	0.13		*NA*	-0.45	*NA*	9	648.069	0.71	0.30	0.069	0.240	18
a)	22	-0.34	0.34	NA		0.03	-0.45	*NA*	9	649.419	2.06	0.16	0.066	0.238	18
	24	-0.34	0.33	0.13		0.03	-0.45	*NA*	10	650.138	2.78	0.11	0.069	0.240	18,20,22
	16	**0.63**	**0.23**	**0.32**			**-0.266**	**+**	**14**	**638.426**	**0**	**0.54**	**0.085**	**0.257**	**---**
	15	0.61	NA	0.33			-0.25	+	13	639.836	1.41	0.27	0.073	0.278	---
b)	8	0.58	0.20	0.31			NA	+	13	642.051	3.63	0.09	0.069	0.232	---
	7	0.57	NA	0.31			NA	+	12	642.700	4.27	0.06	0.059	0.252	---
	14	0.58	0.23	NA			-0.254	+	13	643.947	5.52	0.03	0.063	0.228	---
	53	**2.59**	**NA**	**NA**	**0.169**	**NA**	**-0.226**	**+**	**14**	**4160.572**	**0**	**0.16**	**0.04**	**0.559**	**---**
	49	2.59	NA	NA	NA	NA	-0.231	+	13	4160.895	0.323	0.136	0.039	0.558	---
	61	2.52	NA	NA	0.169	0.13	-0.226	+	15	4161.027	0.455	0.128	0.045	0.561	53,49
	57	2.53	NA	NA	NA	0.13	-0.231	+	14	4161.351	0.779	0.108	0.043	0.56	49
	55	2.59	NA	0.02	0.168	NA	-0.227	+	15	4162.585	2.013	0.059	0.041	0.56	53,49
	54	2.59	0	NA	0.169	NA	-0.226	+	15	4162.703	2.131	0.055	0.045	0.552	53,49
	51	2.60	NA	0.022	NA	NA	-0.231	+	14	4162.866	2.295	0.051	0.04	0.559	49
	50	2.59	-0.01	NA	NA	NA	-0.229	+	14	4162.996	2.424	0.048	0.043	0.551	49
	63	2.52	NA	0.022	0.168	0.13	-0.227	+	16	4163.015	2.444	0.047	0.046	0.563	53,49,61,57,55,51
c)	62	2.52	0.001	NA	0.169	0.13	-0.227	+	16	4163.167	2.595	0.044	0.05	0.554	53,49,61,57,54,50
	59	2.53	NA	0.025	NA	0.13	-0.231	+	15	4163.293	2.721	0.041	0.045	0.562	49,57,51,
	58	2.53	-0.006	NA	NA	0.13	-0.23	+	15	4163.467	2.896	0.038	0.048	0.553	49,57,50
	56	2.59	-0.001	0.02	0.168	NA	-0.227	+	16	4164.725	4.153	0.02	0.046	0.553	53,49,55,54,51,50
	52	2.60	-0.008	0.023	NA	NA	-0.23	+	15	4164.972	4.4	0.018	0.044	0.553	49,51,50
	64	2.52	0	0.022	0.168	0.13	-0.227	+	17	4165.165	4.593	0.016	0.051	0.555	53,49,61,57,55,54, 51,50,63,62,59 58,56,52
	60	2.53	-0.007	0.025	NA	0.13	-0.23	+	16	4165.414	4.843	0.014	0.049	0.555	49,57,51,50,59,58,52
	33	2.708	NA	NA	NA	NA	-0.237	NA	8	4166.19	5.618	0.01	0.037	0.562	---
	37	2.71	NA	NA	0.144	NA	-0.235	NA	9	4166.499	5.927	0.008	0.033	0.551	33

NA: predictors that did not appear in the respective models; **mR²:** marginal R^2 (fixed effects only), **cR²:** conditional R^2 (fixed and random effects), **nested:** models from the candidate set that contain more parameters than a model with a lower AICc value

Variance components of helping behaviours

Animal models are mixed-effect models that decompose phenotypic variance into different sources and are applied to estimate parameters like heritability, maternal effects, or genetic correlations. Using pedigree information, individual breeding values (i.e. the genetic contribution of an individual to a trait compared to the population mean) are estimated and fitted as a random effect in the model. This allows estimating the additive genetic variance V_A, that is, the variance component of breeding values [10]. The Bayesian animal models were run in the MCMCglmm package V 2.22.1 in R, [11]. This necessitates the inclusion of a (typically uninformative) prior. The choice of the most appropriate prior is the topic of on-going debate [12] and currently, there exists no optimal solution. The choice of the prior can potentially affect or bias the results and hence it is advisable to compare the results of models run with different priors. Here, we used two different parameter-expanded priors following suggestions from the Appendix B of [13] and Yimen Araya-Ajoy, pers. comm.). The commonly used inverse-Wishart priors can potentially cause problems with the mixing of the MCMC chain in models with dichotomous response variables [14], especially when the model includes more than two random effects [13]. We used a χ^2 prior (V=1, nu=1000, alpha.mu=0, alpha.V=1) and a scaled Fisher prior (V=1, nu=1, alpha.mu=0, alpha.V=1000) (Appendix B in [13]. The residual variance in binomial models cannot be directly estimated from the data and has to be fixed, usually to 1 [14]. For the Poisson model, we used a similar χ^2-prior, as described above, but estimated the residual variance from the data (V=1, nu=0.002). The priors for the propensities had the following structure (the same specifications were used for all six random effects):

```
priorPropChisq <- list(G=list(G1..6=list(V=1, nu=1000, alpha.mu=0, alpha.V=1),
                        R=list(V=1, fix=1))

priorPropFisher  <-  list(G=list(G1..6=list(V=1,  nu=1,  alpha.mu=0,  alpha.V=1000),
                         R=list(V=10, fix=1))
```

For the amount of defence we used the same specifications for all seven random effects (identity of egg predator was added in the defence models), except for the residual variance (V=1, nu=0.002). We ran threshold models for the propensities and a Poisson model for the amount of defence:

```
MCMCglmm(propensity ~ 1,
random=~animal + motherID + ID + Group + dominantID + Population,
data=data, pedigree=ped, prior=prior, family="threshold",
nitt=2000000, burnin=100000, thin=1000, verbose=FALSE)

MCMCglmm(amount ~ 1,
random=~animal + motherID + ID+ Group + dominantID + Population + eggpredatorID,
data=data, pedigree=ped, prior=prior, family="poisson",
nitt=2000000, burnin=100000, thin=1000, verbose=FALSE)
```

Models were run for 2×10^6 iterations and the first 10^5 were discarded as burn-in to ensure that the chain had converged. The remaining chain was sampled at an interval of 1,000 iterations, which yielded a posterior distribution of the recommended MCMC chain length of >1,000. In total, we ran 12 univariate Bayesian models: four for each helping behaviour, differing in the type of prior used and the inclusion of fixed effects (Table S5). We assessed the quality of models by visually inspecting the trace plots, and checked if the autocorrelation of adjacent samples was <0.1 to see whether draws from the MCMC chain for the posterior distribution were independent. We evaluated the convergence of the chains (Heidelberger diagnostic), and checked if the resulting (effective) sample sizes after correcting for autocorrelation were >1000. The models with the scaled Fisher prior were not converging well and largely failed the Heidelberger diagnostic. Additionally, they had high levels of autocorrelation (up to 0.97) and hence very small effective sample sizes (in some cases below 10). Based on these diagnostics we decided to only use the models run with the χ^2-prior to compute the estimates of heritability and other parameters. Subsequently we computed the observed trait mean, the phenotypic variance, the additive genetic variance and heritability for the whole posterior distribution using the 'QGglmm' package [15].

Table S5: Diagnostics of univariate models for cleaning, digging, and defence.

Name	**link function**	**fixed effects**	**effN***	**conver-gence**	**autocorr**	**marg R^2 ****	**cond R^2 *****
egg cleaning	probit	---	> 1595	passed	< 0.05	---	0.81
egg cleaning	probit	clutch size + submission	> 1632	passed	< 0.03	0.06	0.86
digging	probit	---	> 1685	passed	< 0.02	---	0.70
digging	probit	clutch size + submission + relative size + acceptance status	> 1670	passed#	< 0.06	0.07	0.85
defence	Poisson	---	> 1900	passed	< 0.03	---	0.96
defence	Poisson	submission + egg predator activity + acceptance status	> 1817	passed	< 0.04	0.04	0.96

* effective size of MCMC chain after correction for autocorrelation (should be ≥ 1000)

** marginal R2: variance explained by fixed effects (calculated after a tutorial by Nakagawa)

*** conditional R2: variance explained by the whole model (calculated after a tutorial by Nakagawa)

with the exception of acceptance status 'tolerated'

Results of conditional univariate models and results of repeated runs

Univariate models including fixed effects yielded similar estimates of the relevant parameters as intercept-only models (Table S6). This was not unexpected because the marginal R^2 of those models were very small, indicating that the fixed effects explained only a small proportion of the variance (Table S5). We ran three additional intercept-only models with the χ^2-prior for each behaviour to ensure that the results were stable. These resulted in relatively similar estimates (Table S7) except for the maternal effect of digging propensity that had a bimodal posterior distribution with one mode at zero and one at approximately 10%. This led to a posterior mode of 0.107 (2×10^{-6}, 0.214) in one case

and 0.010 (5.5×10^{-6}, 0.218) and 0.001 (1.6×10^{-6}, 0.223) in other cases. Thus, we consider the models generally robust and the majority of estimates stable.

Table S6: Estimates of quantitative genetics parameters derived from conditional models (including covariates as fixed effects) using the QGglmm package [15]. In the conditional models, we included the covariates as fixed effects that were identified as important predictors of the helping behaviour in a previous model selection step (see main text and Table S4).

	egg cleaning	**digging**	**defence**
fixed effects in model	**clutch size + submission**	**clutch size + relative size + submission + acceptance status**	**average egg predator activity + submission + acceptance status**
trait mean	0.437 (0.414, 0.453)	0.530 (0.516, 0.536)	34.457 (27.520, 49.644)
phenotypic variance (V_P)	0.246 (0.243, 0.248)	0.249 (0.249, 0.25)	1.6×10^{3}(2.13×10^{3},2.78×10^{3})
add. genet. variance (V_A)	4.5×10^{-5} (9.2×10^{-13}, 0.024)	3.5×10^{-4} (5.1×10^{-9}, 0.060)	0.001 (2.7×10^{-9}, 1.460)
heritability (h^2)	2.2×10^{-4} (3.7×10^{-12}, 0.098)	0.001 (2.1×10^{-8}, 0.239)	1.3×10^{-4} (4.8×10^{-10}, 0.024)
permanent environment effect (V_{PE})	0.503 (0.229, 0.711)	0.003 (2.6×10^{-6}, 0.457)	6.0×10^{-4} (3.1×10^{-10}, 0.084)
repeatability (R)	0.626 (0.359, 0.778)	0.555 (0.225, 0.738)	0.354 (0.220, 0.535)
maternal effect (V_M)	0.001 (4.1×10^{-9}, 0.125)	0.001 (2.8×10^{-6}, 0.213)	0.281(0.139, 0.417)
common environment effect (V_{CE})	4.8×10^{-4} (9.9×10^{-9}, 0.076)	4.3×10^{-4} (2.4×10^{-9}, 0.111)	3.5×10^{-4} (3.9×10^{-9}, 0.068)
social effect (V_S)	0.001 (1.3×10^{-11}, 0.145)	0.001 (9.7×10^{-8}, 0.193)	1.4×10^{-4} (6.4×10^{-8}, 0.026)
egg predator identity effect (V_{VID})	---	---	0.114 (0.034, 0.319)

Table S7: Repeated runs of univariate models without fixed effects to demonstrate stability of the models reported in Table 1 in the main text across runs using the identical model structures (see 'Variance components of helping behaviour' above).

egg cleaning	model 1	model 2	model 3
trait mean	0.419 (0.36, 0.518)	0.432 (0.358, 0.513)	0.427 (0.354, 0.514)
phenotypic variance (V_P)	0.25 (0.234, 0.25)	0.25 (0.235, 0.25)	0.25 (0.233, 0.25)
add. genet. variance(V_A)	9.0×10^{-5} (4.4×10^{-12},	1.4×10^{-4} (7.7×10^{-10}, 0.033)	2.5×10^{-4} (4.0×10^{-9}, 0.031)
heritability (h^2)	4.3×10^{-4} (1.2×10^{-11}, 0.133)	0.001 3.3×10^{-9}, 0.133)	0.001 (1.7×10^{-8}, 0.126)
permanent environment effect (V_{PE})	0.463 (0.160, 0.620)	0.342 (0.140, 0.624)	0.430 (0.146, 0.621)
repeatability (R)	0.574 (0.288,0.715)	0.527 (0.283, 0.735)	0.482 (0.292, 0.734)
maternal effect (V_M)	0.001 (8.7×10^{-8}, 0.136)	0.001 (3.8×10^{-8}, 0.132)	0.001 (4.3×10^{-9}, 0.128)
common environment effect (V_{CE})	0.34 (0.156, 0.616)	0.391 (0.130, 0.614)	0.330 (0.142, 0.619)
social effect (V_S)	4.4×10^{-4} (2.8×10^{-9}, 0.085)	4.8×10^{-3} (8.6×10^{-10}, 0.087)	4.0×10^{-4} (1.0×10^{-10}, 0.086)
maternal effect (V_M)	4.3×10^{-4} (1.2×10^{-9}, 0.106)	0.001 (9.3×10^{-9}, 0.103)	0.001 (1.0×10^{-8}, 0.108)
digging	**model 1**	**model 2**	**model 3**
trait mean	0.518 (0.445, 0.599)	0.526 (0.447, 0.599)	0.515 (0.449, 0.6)
phenotypic variance (V_P)	0.25 (0.242, 0.25)	0.25 (0.243, 0.25)	0.25 (0.242, 0.25)
add. genet. variance(V_A)	1.5×10^{-4} (6.0×10^{-10},	2.5×10^{-4} (4.3×10^{-7}, 0.052)	2.7×10^{-4} (7.4×10^{-9}, 0.051)
heritability (h^2)	0.001 (2.4×10^{-9}, 0.209)	0.001 (1.7×10^{-6}, 0.208)	0.001 (3.0×10^{-8}, 0.205)
permanent environment effect (V_{PE})	0.002 (9.8×10^{-7}, 0.270)	0.002 (2.6×10^{-8}, 0.268)	0.002 (3.8×10^{-7}, 0.272)
repeatability (R)	0.296 (0.130, 0.537)	0.335(0.140, 0.540)	0.284 (0.131, 0.537)
maternal effect (V_M)	0.107 (2×10^{-6}, 0.214)	0.001 (1.6×10^{-6}, 0.223)	0.010 (5.6×10^{-6}, 0.218)
common environment effect (V_{CE})	0.002 (9.5×10^{-7}, 0.243)	0.002 (2.4×10^{-8}, 0.243)	0.001 (3.4×10^{-7}, 0.250)
social effect (V_S)	3.8×10^{-4} (1.2×10^{-9}, 0.083)	3.7×10^{-4} (2.5×10^{-8}, 0.082)	1.9×10^{-4} (2.0×10^{-8}, 0.078)
maternal effect (V_M)	6.6×10^{-4} (3.7×10^{-8}, 0.089)	2.3×10^{-4} (9.1×10^{-10}, 0.091)	2.3×10^{-4} (6.4×10^{-9}, 0.092)
defence	**model 1**	**model 2**	**model 3**
trait mean	36.769 (20, 66.216)	33.573 (17.895, 64.139)	33.65 (18.869, 64.41)
phenotypic variance (V_P)	5068.032 (1197.77,	852.516 (1224.663,	478.265 (849.749, $4.17*10^4$)
add. genet. variance(V_A)	7.291 (1.7×10^{-4}, 512.132)	54.042 (3.3×10^{-5}, 532.035)	34.559 (1.7×10^{-4}, 492.469)
heritability (h^2)	2.2×10^{-4} (2.5×10^{-8}, 0.047)	2.8×10^{-4} (2.6×10^{-9}, 0.047)	3.1×10^{-4} (2.3×10^{-8}, 0.046)
permanent environment effect (V_{PE})	4.6×10^{-4} (6.8×10^{-8}, 0.076)	4.4×10^{-4} (2.2×10^{-10}, 0.074)	4.5×10^{-4} (4.3×10^{-11}, 0.076)
repeatability (R)	0.325 (0.199, 0.489)	0.338 (0.183, 0.476)	0.350 (0.200, 0.499)
maternal effect (V_M)	0.238 (0.122, 0.384)	0.228 (0.128, 0.395)	0.262 (0.116, 0.389)
common environment effect (V_{CE})	4.6×10^{-4} (6.8×10^{-8}, 0.076)	4.4×10^{-4} (2.2×10^{-10}, 0.074)	4.5×10^{-4} (2.3×10^{-10}, 0.076)
social effect (V_S)	1.9×10^{-4} (5.0×10^{-8} 0.068)	2.1×10^{-4} (8.1×10^{-9}, 0.063)	3.9×10^{-4} (2.6×10^{-9}, 0.065)
maternal effect (V_M)	7.2×10^{-5} (2.2×10^{-8}, 0.068)	1.4×10^{-4} (2.7×10^{-9}, 0.028)	8.6×10^{-5}(2.4×10^{-8}, 0.068)
egg predator identity effect (V_{VID})	0.146 (0.053, 0.357)	0.150 (0.051, 0.348)	0.137 (0.052, 0.343)

Assessing statistical importance

Density plots of posterior distributions

We evaluated the statistical importance of the variance component estimates (posterior distributions of the model parameters) as well as the derived measures of heritability, repeatability etc. by visually assessing the shapes of the posterior distributions. Since the variance (and the proportion of variance explained) is constrained to be positive, the non-overlap of the credibility interval with 0 cannot be interpreted as a sign of statistical significance. Thus, we inspected the shape of the posterior distribution and considered those effects as not statistically significant that had a zero-truncated

posterior distribution, i.e. a large function value at zero. The posterior distributions of the additive genetic variances of all behaviours were definitely zero-truncated.

Percentage of non-overlap with null models

We compared the real posterior distribution of each model parameter to ten null distributions (as suggested by Y. Araya-Ajoy, pers. comm.). Each null distribution was obtained by permuting the values of the variable, and performing a model on this permuted variable. Then, for each variance component we calculated the difference of the real with each the null distribution. The percentage of non-overlap of the real with the null posterior distributions were calculated by taking the proportion of all the differences that are greater then zero, i.e. the cases in which the real posterior value was larger than the posterior value of the null distribution. Overlap of the real distributions with the null distributions was very low (< 2%, Table S8) for the permanent environment (ID random effect) of egg cleaning, the maternal effect (mother ID) and the egg predator effect (*T. vittatus* ID), and can thus be considered as statistically important.

Table S8: Percentage of non-overlap of the real posterior distributions with ten null distributions. model.1: intercept-only models, model.2: conditional models

	cleaning.1	**cleaning.2**	**digging.1**	**digging.2**	**defence.1**	**defence.2**
add. genet. variance(V_A)	78.41	72.19	81.50	85.61	61.01	63.65
maternal effect (V_M)	86.38	84.82	94.42	90.31	**99.99**	**100.00**
permanent environment (V_{PE})	**98.26**	**98.51**	54.31	66.89	41.94	29.10
common environment (V_{CE})	60.17	68.07	55.88	65.24	53.41	60.13
social effect (V_S)	58.94	72.63	49.18	63.15	32.64	24.84
Population (V_{Pop})	74.71	66.35	59.58	69.77	81.98	83.94
egg predator identity (V_{VID})	---	---	---	---	**99.85**	**97.81**

Phenotypic, genetic, and maternal correlations

Estimates of genetic correlations with other traits provide insight into whether multiple helping traits have a shared genetic basis and coevolve [20]. It was technically not possible to calculate the covariances of all three behaviours due to the high demands on statistical power of these multivariate models. Pairwise genetic, maternal and residual correlations between the helping behaviours were estimated by specifying separate bivariate Bayesian animal models for the amount of defence and digging propensity, and for the amount of defence and cleaning propensity. We ran the models with a prior with a variance of one for each behaviour and each variance component as well as the residual, and a degree-of-belief parameter equal to the dimensions of the variance-covariance matrix. We estimated the covariances between all traits and fixed the residual variance of the propensities to 1. To ensure acceptable convergence and posterior distribution lengths, we ran the models for 9.9×10^6 iterations after a burn-in phase of 10^5 and sampled at intervals of 5,000. We used a prior with a variance of 1 for each behaviour and each variance component as well as the residual, and a degree-of-belief parameter equal to the dimensions of the variance-covariance matrix (2):

```
priorMV <- list(G=list(
             G1=list(V=diag(2),nu=2,     alpha.mu=c(0,0),     alpha.V=diag(2)*1000),
             G2=list(V=diag(2),nu=2,     alpha.mu=c(0,0),     alpha.V=diag(2)*1000)),
             R=list(V=diag(2),nu=2, fix=2))


Corr1 <- MCMCglmm(cbind(Df,Dg) ~ trait-1,
                random=~us(trait):animal + us(trait):DamID,
                rcov=~us(trait):units,
                data=dataNR, pedigree=ped,
                family=c("poisson","threshold"), verbose = F, prior=priorMV,
                nitt= 9900000, thin=5000, burnin=4100000)

Corr2 <- MCMCglmm(cbind(Df,Cl) ~ trait-1,
                random=~us(trait):animal + us(trait):DamID,
                rcov=~us(trait):units,
                data=dataNR, pedigree=ped,
                family=c("poisson","threshold"), verbose = F,  prior=priorMV,
                nitt=9900000, thin=5000, burnin=100000)
```

The model for the covariances of defence and digging did not converge, even after increasing the burn-in, and autocorrelation was high, resulting in low effective sample sizes. We therefore only report the results for the second model (defence and cleaning), which produced acceptable diagnostics. Using a similar method as described above we transformed the covariances from the latent scale to the data scale and calculated the correlation coefficients of the phenotypic, genetic and maternal correlations as the standardized covariances: $r_{A,B} = \frac{cov_{A,B}}{\sqrt{var_A * var_B}}$ [21]. In addition to visually assessing the shapes of posterior distributions we considered correlations as statistically important when their credibility intervals did not include zero. Egg cleaning and defence against the egg predator were positively correlated on the phenotypic level, but, in line with the lack of any additive genetic variance underlying any of these behaviours, additive genetic correlations were poorly estimated and non-significant (Table S9). We find a significant positive maternal correlation between egg cleaning and defence.

Table S9: Phenotypic, additive genetic (G), and maternal variance-covariance matrices of helping behaviour determined by a bivariate Bayesian animal model, using the QGmvparams and the QGmvicc functions in the QGglmm package. Variances are shown in the diagonal, covariances in the upper triangle and correlations in the lower triangle (estimates with 95% credibility intervals). Correlation estimates in bold indicate statistical importance (credible intervals do not include zero).

	Defence	**egg cleaning**	**defence**	**egg cleaning**
	phenotypic		**additive genetic**	
defence	4813.752 (1834.496, 17253.432)	4.285 (2.236, 8.282)	10.581 (0.002, 2019.197)	-0.004 (-0.771, 5.831)
egg cleaning	**0.129 (0.068, 0.166)**	0.246 (0.239, 0.250)	0.866 (-0.670, 0.996)	0.000 (6.79×10^{-09}, 0.0346)

	maternal	
defence	1226.929 (141.392, 2938.506)	2.470 (-0.270, 4.270)
egg cleaning	**0.736 (0.230, 0.885)**	0.008 (0.000, 0.020)

Effects of population-level predator densities on helping behaviours

We found an effect of maternal identity on the amount of defence an individual performs. In the case of adaptive maternal effects, mothers transmit relevant information about the environment they experience to the next generation, for example about predation threat [22,23]. The mothers of the test subjects were caught as adults at six distinct populations in Lake Tanganyika and then brought to our animal keeping facility where the populations were kept separately but under identical conditions in mixed-sex tanks. We used data obtained by [1] on the number of egg and fish predators per transect at the locations where the females were caught. The amount of defence shown by the test fish neither correlated with the egg predation risk (Spearman's Rho = 0.037, p = 0.414) nor the fish predation risk in their parents' population (Spearman's Rho = -0.044, p = 0.330). Mixed-effect Poisson models including either egg or fish predation threat (additionally to the fixed and random effects described in the Model selection section) corroborated the results of the correlations. Neither egg nor fish predation risk were significant predictors of defence (LRT: χ^2=0.22, df=1, p=0.641, and χ^2=1.57, df=1, p=0.210, respectively). Thus, offspring of mothers that were caught at locations with high egg or fish predation risk did not differ in their defence behaviour from those whose mothers came from low-predation risk areas. Also, including egg or fish predation risk in a univariate Bayesian animal model did not change the estimate of the maternal effect as compared to the models outlined above. The maternal effect on digging was not so clear, with some runs of the same model resulting in a significant maternal effect, whereas others did not. However, we did similar checks for the influence of rock:sand ratio in the mother's population on digging propensity as we did for predation risk on defence. We also find no correlation of digging propensity with sandiness (Spearman's Rho = -0.027, p = 0.557), which was confirmed by a GLMM as described above (LRT: χ^2=0.328, df=1, p=0.567).

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